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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

APR 15 1997

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Pirimiphos-methyl. List B Reregistration Case No. 2535/Chemical ID No. 108102. Nature of the Residue in Stored Grain. MRID Nos. 42903504 and 42903501. CBRS No. 12557. DP Barcode No. D195119.

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THRU: Randolph B. Perfetti, Ph.D., Acting Branch Chief
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TO: Jane Smith
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In response to reregistration data requirements, Wilbur-Ellis Co. has submitted a ¹⁴C-pirimiphos-methyl metabolism study in stored corn grain treated postharvest, prior to placement in storage. The study has been reviewed by Dynamac under supervision of CBRS, and has been revised to reflect current Agency policy.

The study is adequate, and the nature of the residue in stored grain is adequately understood. The residues of concern in stored grain include the parent, pirimiphos methyl, and its metabolite O-[2-ethylamino-6-methyl-pyrimidin-4-yl] O,O-dimethyl phosphorothioate. The residues to be regulated in the tolerance expression will be determined upon completion of review of submitted livestock metabolism studies.

cc: CSwartz; Circulate; SF; List B File; RF; Ron Kendall/Kathleen Depukat (PM 51, SRRD, 7508W)

7509C:CSwartz:CBRS:CM2,Rm.804F:703 305 5877:4/8/97

RDI:RBPerfetti:4/10/97



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PIRIMIPHOS-METHYL

Shaughnessy No. 108102; Case 2535

(CBRS No. 12557; DP Barcode D195119)

Registrant's Response to Residue Chemistry Data Requirements

March 18, 1997

Contract No. 68-D4-0010

**Submitted to:
U.S. Environmental Protection Agency
Arlington, VA**

**Submitted by:
Dynamac Corporation
1910 Sedwick Road
Building 100, Suite B
Durham, NC 27713**

PIRIMIPHOS-METHYL

Shaughnessy No. 108102; Case 2535

(CBRS No. 12557; DP Barcode D195119)

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

The Pirimiphos-methyl Phase IV Review (1/91) required a new plant metabolism study. In response, Wilbur-Ellis Company, through their agent Compliance Services International, submitted a [^{14}C]pirimiphos-methyl metabolism study on corn (1993; MRID 42903501); an additional volume (MRID 42903504) contained data on characterization of the test substance and reference standards used in metabolism studies. These data are reviewed herein for adequacy in fulfilling residue chemistry data requirements.

Tolerances are established (40 CFR §180.409) for pirimiphos-methyl and its metabolites O-[2-ethylamino-6-methyl-pyrimidin-4-yl] O,O-dimethyl phosphorothioate and, in free and conjugated form, the metabolites 2-diethylamino-6-methyl-pyrimidin-4-ol, 2-ethylamino-6-methyl-pyrimidin-4-ol, and 2-amino-6-methyl-pyrimidin-4-ol in corn (8.0 ppm), sorghum grain (8.0 ppm), kiwifruit (5.0 ppm), eggs (0.5 ppm), milk fat (3.0 ppm; 0.1 (N) in whole milk); fat of cattle, goats, hogs, horses, poultry, and sheep at 0.2 ppm; kidney and liver of cattle, goats, hogs, horses, and sheep at 2.0 ppm; meat and meat byproducts of cattle, goats, hogs, horses, and sheep at 0.2 ppm; and meat and meat byproducts of poultry at 2.0 ppm. Food and feed additive tolerances have been established under 40 CFR §185.4950 and §186.4950 for corn milling fractions (except flour) and sorghum milling fractions (except flour) at 40 ppm and under 40 CFR §185.4950 for corn oil at 88 ppm. The tolerances for residues in corn and sorghum grain were established for postharvest application to stored grain.

CONCLUSIONS

1. Corn grain was treated with [^{14}C]pirimiphos-methyl to simulate the registered application to grains prior to placement in storage. Three successive applications were made at 28 mg ai/600 g of grain per application, equivalent to 2.8 lb ai/30 tons (6X the maximum registered rate). Grain samples were collected on the day of application and after 12 and 24 weeks of storage at ambient temperature in a desiccator.
2. Total radioactive residues (TRRs) were determined via combustion of whole grain followed by liquid scintillation spectroscopy (LSS) of the trapped $^{14}\text{CO}_2$. Total radioactive residues were also determined in MeOH extracts and in bound fractions.

3. Total radioactive residues in the zero day corn samples ranged from 86.8 to 110.9 ppm. At 12 weeks after treatment (Group 2) radioactivity had decreased to 50.4-53.3 ppm and after 24-weeks (Group 3) total radioactive residues were 43.1-45.3 ppm.
4. Radioactive residues were extracted from corn grain with methanol (MeOH). The remaining insoluble fraction was acid hydrolyzed, and residues in the hydrolysate partitioned into hexane, dried and reconstituted in MeOH. Additional extractions and cleanup were performed on the hydrolyzed insoluble fractions from some samples, but did not result in further characterization of unidentified radioactivity.
5. Radioactivity in corn grain extracts was identified via co-elution with non-radiolabeled standards using high performance liquid chromatography (HPLC) with a gradient solvent system. Identification of pirimiphos-methyl metabolites was confirmed using thin layer chromatography (TLC).
6. The major residue in corn grain treated postharvest with [^{14}C]pirimiphos-methyl was the parent, pirimiphos-methyl *per se*, which constituted >96% of the TRR in zero-time samples and 64-67 %TRR in 12 and 24 week samples. Metabolites A and C were the only metabolites detected in zero day samples, each at $\leq 2.4\%$ of the TRR. Metabolites A and C were found in 12 week samples at up to 10.3 %TRR, with metabolites D and E/F present at up to 3.2 %TRR. Unidentified radioactivity in 12-week samples totalled 14.5 %TRR, but with no one component at >2 %TRR. In 24-week samples, metabolite C constituted up to 22 %TRR, metabolite D was identified at up to 8.6 %TRR, and metabolites E/F were present at ≤ 0.2 %TRR.
7. The corn grain metabolism study is adequate. The nature of the residue in stored grain is understood; principal residues consist of the parent, pirimiphos-methyl, and its metabolite O-[2-ethylamino-6-methyl-pyrimidin-4-yl) O,O-dimethyl phosphorothioate (Metabolite A, R36341).

Figure 1. Chemical names and molecular structures of pirimiphos-methyl and its metabolites in plants and animals.

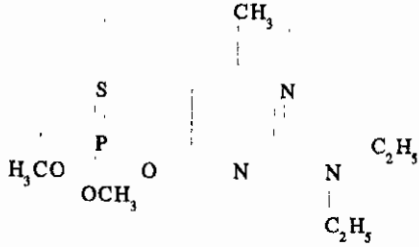
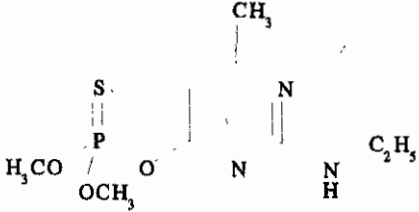
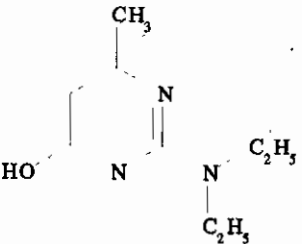
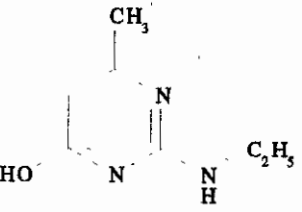
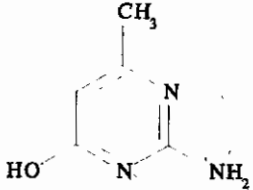
Pirimiphos-Methyl Common Name	Structure
O-(2-diethylamino-6-methylpyrimidin-4-yl)-O,O-dimethyl phosphorothioate Pirimiphos-methyl	
O-(2-ethylamino-6-methylpyrimidin-4-yl)-O,O-dimethyl phosphorothioate Compound A R36341, des-ethyl pirimiphos-methyl, DPM	
2-diethylamino-6-methyl-pyrimidin-4-ol Compound C R46382	
2-ethylamino-6-methyl-pyrimidin-4-ol Compound D R35510	
2-amino-6-methyl-pyrimidin-4-ol Compound E R4039	

Figure 1. continued

Pirimiphos-Methyl Common Name	Structure
2-hydroxy-6-methyl-pyrimidin-4-ol	
Compound F	

DETAILED CONSIDERATIONS

Application of the Test Substance

Compliance Services International, on behalf of Wilbur-Ellis Co., (1993; MRID 42903501) submitted data from a corn grain metabolism study on pirimiphos-methyl. The test substance [2-¹⁴C]pirimiphos-methyl was diluted with non-labeled pirimiphos-methyl to a final specific activity of 10.04 μ Ci/mg; radiochemical purity was 98% with 2% Compound A present as an impurity.

Two 600 g samples of corn grain (Groups 2 and 3) were slow-dried at 37-45 C to a moisture content of 14%. Grain samples were spread on a tray and sprayed with 4 mL of the dosing emulsion containing 28 mg ai [¹⁴C]pirimiphos-methyl. Treated grain was allowed to dry for 1 hour then placed in a bag and mixed for 1 minute. The treatment was then repeated twice. The rate applied in each of the three treatments was equivalent to 6X the registered single application rate. A third 689 g grain sample (Group 1) served as a control.

Samples were placed in a desiccator, and sub-samples were collected on the day of treatment; Group 2 and Group 3 grain samples were collected 12 and 24 weeks after treatment, respectively, and were stored frozen until analysis (within 5 months of collection). Analyses were conducted by WIL Research Laboratories, Inc., Ashland, OH.

Total radioactive residues (TRR)

Total radioactive residues in each sample were determined by direct radioassay of the initial extract by liquid scintillation spectroscopy (LSS) and LSS of the extracted solid fraction following oxidation. Whole grain from the 12- and 24-week samplings were also radioassayed by LSS following oxidation; the results from these determinations were in agreement with those obtained following extraction. The TRR in treated grain is summarized in Table 1. Total radioactive residues in the zero day samples ranged from 86.8 to 110.9

ppm. At 12 weeks after treatment (Group 2) TRR had decreased to 50.4-53.3 ppm and after 24-weeks (Group 3) TRR were 43.1-45.3 ppm.

Table 1. Total Radioactive Residues in Corn Grain.

Sampling interval (weeks)	TRR (ppm) ^a
0 (Group 2)	94.7 93.0 86.8 Average = 91.5
0 (Group 3)	93.8 110.9 88.8 Average = 97.8
12 (Group 2)	50.4 53.3 50.7 Average = 51.5 <i>50.0 50.8 50.3</i>
24 (Group 3)	43.1 45.3 43.8 Average = 44.1 <i>38.0 46.8 53.5</i>

^a Sum of radioactivity in first MeOH extract and extracted grain; values in *italics* are the results from combustion LSS of whole grain samples, not yet extracted.

Extraction and hydrolysis of ¹⁴C-residues

Radioactive residues were extracted from corn grain with methanol (MeOH). The remaining insoluble fraction was acid hydrolyzed using 2 M HCl (50% in MeOH) for 20 minutes. The hydrolysate was partitioned with hexane, the aqueous fraction was evaporated, and the residues were reconstituted in MeOH. The two MeOH fractions were analyzed using HPLC and TLC. Additional extractions and cleanup were performed on the hydrolyzed insoluble fractions from some samples; however, these did not yield any additional fractions that were analyzed by HPLC or TLC. The results of residue extraction and analysis are summarized in Table 2. Since three samples were analyzed from each sampling, results are reported as ranges. Only data from Group 2 zero-time samples are reported here, since Group 3 zero-time samples exhibited the same residue distribution. Methanol extracted >99% of the radioactivity from zero-day samples and 93.6-94.6% from the 12- and 24-week stored samples. The proportion of bound residues increased slightly after 12 weeks to >5%. Less than half of the insoluble residues were released by subsequent acid hydrolysis.

Table 2. Extraction of [¹⁴C]pirimiphos-methyl residues from Corn Grain.

Fraction	% TRR	ppm	Analysis
Corn Grain (Group 2) Zero Time (86.8-94.7 ppm)			
MeOH I	99.0-99.3	85.8-94.0	HPLC: Pirim-methyl 96.2-97.3 % 83.3-92.0 ppm Metabolites: A: 1.5-2.4 %, 1.4-2.1 ppm C: ND-0.7 %, ND-0.7 ppm

Table 2. (continued)

Table 2. Extraction of [¹⁴C]pirimiphos-methyl residues from Corn Grain.

Fraction	% TRR	ppm	Analysis
Insoluble	0.69-0.95	0.75-0.96	Acid hydrolysis: 2 M HCl 50% in MeOH, 20 min; hexane partition, evaporate, reconst in MeOH
MeOH II	0.21-0.33	0.23-0.33	HPLC: Metabolites: C: 0.2%, 0.17-0.28 ppm Unknowns: 0.1%, 0.05-0.11 ppm
Insoluble II	0.36-0.48	0.40-0.48	Further analysis did not yield additional information
Corn Grain (Group 2) 12 Weeks (50.4-53.3 ppm)			
MeOH I	94.1-94.6	47.6-50.2	HPLC: Pirim-methyl 64.3-72.7% 32.6-36.7 ppm Metabolites: A: 2.7-10.3%; 1.4-5.2 ppm C: 4.4-9.2%; 2.2-4.7 ppm D: 0-3.1%, 0-1.6 ppm E/F: 0.7-2.4%, 0.4-1.2 ppm Unknowns: 4.5-14.4%, 2.3-7.3 ppm
Insoluble I	5.41-5.90	2.74-3.15	Acid hydrolysis: 2 M HCl 50% in MeOH, 20 min; hexane partition, evaporate, reconst in MeOH
MeOH II	1.3-1.7	0.64-0.90	HPLC: Metabolites: C: 0.9-1.1%, 0.46-0.58 ppm D: 0.1%, 0.50-0.11 ppm E/F: 0.1-0.2%, 0.03-0.08 ppm Unknowns: 0.1-0.3%, 0.05-0.13 ppm
Insoluble II	3.6-3.8	1.81-2.02	Further analysis did not yield additional information
Corn Grain (Group 3) 24 Weeks (43.1-45.3 ppm)			
MeOH I	93.6-94.1	40.1-41.9	HPLC: Pirim-methyl 64.4-67.4% 27.5-30.2 ppm Metabolites: C: 18.7-21.5%, 8.4-9.2 ppm D: 8.0-8.4%, 3.5-3.8 ppm
Insoluble I	5.92-6.40	3.02-3.43	Acid hydrolysis: 2 M HCl 50% in MeOH, 20 min; hexane partition, evaporate, reconst in MeOH

Table 2: (continued)

Table 2. Extraction of [^{14}C]pirimiphos-methyl residues from Corn Grain.

Fraction	% TRR	ppm	Analysis
MeOH II	0.95-1.10	0.48-0.56	HPLC: Metabolites: C: 0.5-0.8%, 0.25-0.38 ppm D: 0.1-0.2%, 0.05-0.09 ppm E/F: 0.1-0.2%, 0.02-0.07 ppm Unknowns: 0.2-0.3%, 0.07-0.14 ppm
Insoluble II	4.7-5.0	2.3-2.7	Further analysis did not yield additional information

Analytical Methods Used to Characterize/Identify ^{14}C -residues

Radioactive residues in MeOH fractions of corn grain were identified via co-elution with non-radiolabeled standards using HPLC (System 2) with a reverse phase C18 column and a linear gradient from 100% solvent A (water:mobile phase B, 99:1) to 10% solvent A/90% solvent B (0.01 M ammonium acetate:acetonitrile, 10:90). Radioactive peaks were located and quantitated using a radiochemical detector. Non-radiolabeled standards were visualized using UV detection. Metabolite structures are shown in Figure 1. Fractions corresponding to metabolites E and F, which were not resolved by HPLC system 2, were collected, cleaned up on silica gel and analyzed via HPLC using a normal phase silica gel column and an isocratic gradient of ACN:H₂O (60:40) (HPLC system 3). In most cases, however, HPLC system 3 did not provide peaks for E and/or F that were quantifiable above background.

To confirm identities of metabolites, extracts were spotted onto 2-dimensional silica gel TLC plates and developed in hexane:acetone (10:1) and chloroform:methanol (85:15). Migration of compounds was analyzed by fluorescence quenching under UV light. Radioactive zones were quantitated using a plate scanner. Compounds were identified by comparison with reference standards.

Results

Results of attempts to identify or characterize ^{14}C -residues in corn grain are presented in Table 2 for each fraction analyzed. The percentages and concentrations of metabolites in each matrix are summarized in Table 3. The major ^{14}C -residue in [^{14}C]pirimiphos-methyl treated corn grain was the parent, pirimiphos-methyl *per se*, which constituted >96% of the TRR in zero-time samples and 64-67 %TRR in 12 and 24 week samples. Metabolites A and C were the only metabolites detected in zero day samples, each at $\leq 2.4\%$ of the TRR. Concomitant with the decrease in the parent after 12 and 24 weeks of storage, the levels of metabolites increased. Metabolites A and C were found in 12 week samples at up to 10.3 %TRR, with metabolites D and E/F present at up to 3.2 %TRR. Unidentified radioactivity

in 12-week samples totalled 14.5 %TRR, but with no one component at >2 %TRR. In 24-week samples, metabolite C constituted up to 22 %TRR, metabolite D was identified at up to 8.6 %TRR, and metabolites E/F were present at ≤ 0.2 %TRR.

Table 3. Identification/Characterization of [^{14}C]pirimiphos-methyl residues in corn grain.

Sampling interval	Zero time	12 weeks	24 weeks
Metabolite/fraction	Percent TRR in matrix (ppm in parentheses)		
Pirimiphos-methyl	96.2-97.3 (83.3-92.0)	64.3-72.7 (32.6-36.7)	64.4-67.4 (27.5-30.2)
Compound A	1.5-2.4 (1.4-2.1)	2.7-10.3 (1.4-5.2)	ND ^a
Compound C	ND-0.9 (ND-0.9)	5.3-10.2 (2.7-5.2)	19.3-22.1 (8.7-9.4)
Compound D	ND	0.1-3.2 (0.05-1.7)	8.1-8.6 (3.5-3.9)
Compound E/F	ND	0.8-2.5 (0.5-1.2)	<0.1-0.2 (0.02-0.07)
HPLC unknowns ^b	0.1 (0.05-0.1)	4.8-14.5 (2.4-7.4)	0.2-0.3 (0.07-0.14)
Insoluble	0.4-0.5 (0.4-0.5)	3.6-3.8 (1.8-2.0)	4.7-5.0 (2.3-4.7)
Total identified/characterized	99.2-99.9	99.3-99.5	99.5-100

^a ND = Not detected.

^b Numerous unknowns, each constituting <5 %TRR.

CBRS Comment

The metabolism study in stored grain is adequate. Grain treated post-harvest and stored for 12 to 24 weeks exhibited only slight to moderate metabolism/degradation of pirimiphos-methyl, which persisted at >64% of the TRR after 24 weeks. Metabolite A, the only organophosphate metabolite, was present in 12-week stored samples at up to 10% of the TRR; this metabolite was not detected after 24 weeks. The remaining hydroxypyrimidine metabolites did not exceed 10% of the TRR in any sample with the exception of 24-week samples, which contained metabolite C at 19-22%. The residue of concern in stored grain is the parent, pirimiphos-methyl, and its metabolite O-(2-ethylamino-6-methylpyrimidin-4-yl)-O,O-dimethyl phosphorothioate (metabolite A).

MASTER RECORD IDENTIFICATION NUMBER

42903501 Hauswald, C. (1993) The Fate of (Carbon 14)-Pirimiphos-Methyl on Stored Corn Grain; Final Report: Lab Project Number: WECO-9205. Unpublished study prepared by WIL Research Labs., Inc. 416 p.

42903504 Hauswald, C.(1993) Method Development and Purification/Characterization of Reference Compounds for Pirimiphos-Methyl Metabolism Studies; Final Report: rain: Laboratory Study Metabolism Studies; Final Report: Lab Project Number: WIL-205001: WECO-9210. Unpublished study prepared by WIL Research Labs., Inc. 157 p.